

IMMUNE 3D®

A New Dimension to 3D Co-Cultures

Simple and Efficient Platform for IO Drug Discovery

Dynamic Early Stage Company

Established in 2015 and based in Rockville, MD

Proprietary and Modular 3D Platform

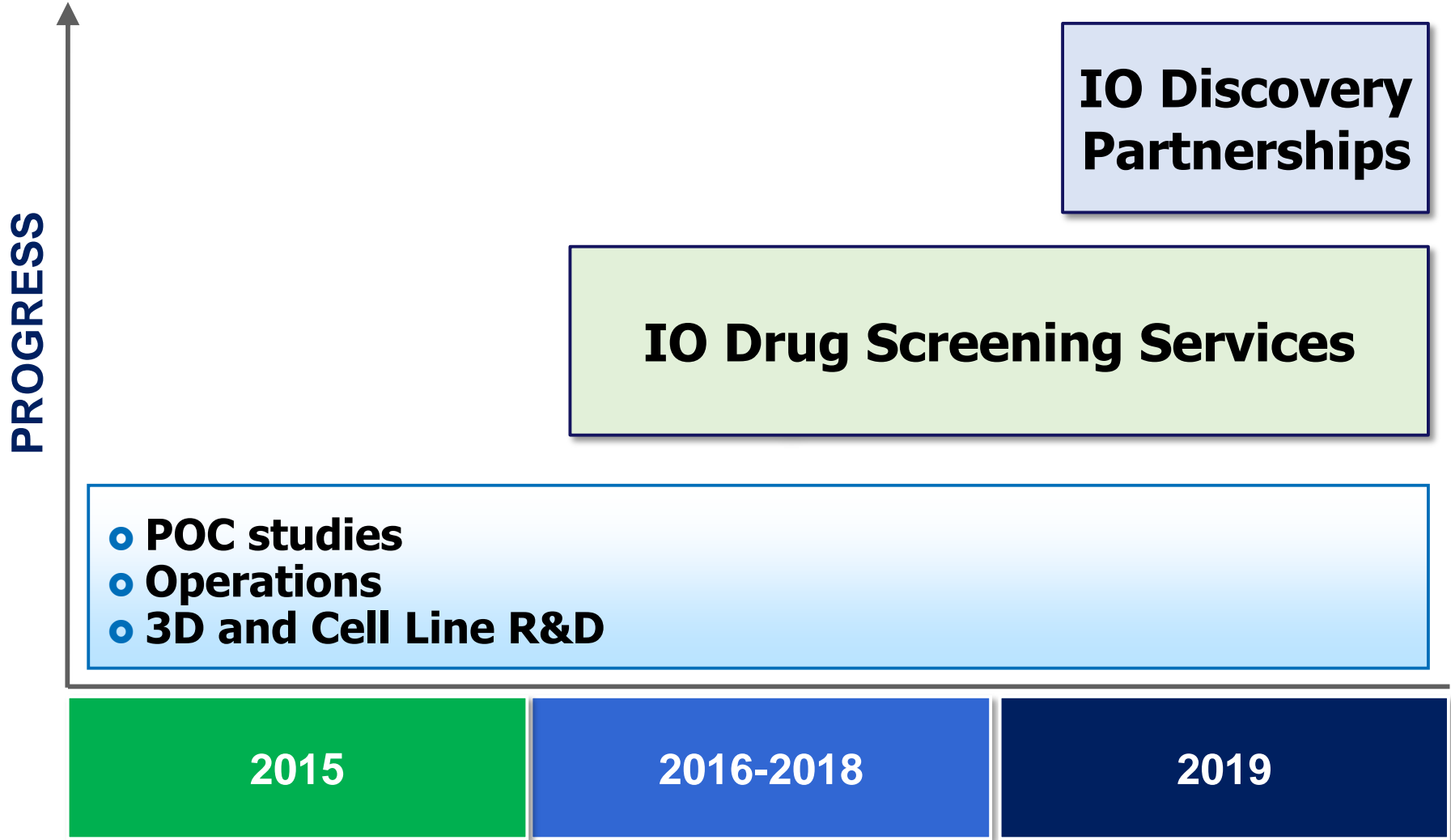
Study Key Factors of the Tumor Immune Microenvironment (TIME)

Access to Human and Patient-Derived Cells

Well Characterized Cell Platforms = Valuable "Inputs"

Innovative, Nimble and Experienced

Team of Business, Scientific and Clinical partners



- Important for **mounting effective immune responses.**
- Permits **T cell to recognize antigen** presented in the context of MHC by an antigen presenting cells and then traffic and interact with other cells.
- In IO, several groups demonstrate that the presence of T cells cross multiple tumor types within the tumor microenvironment is **predictive of response to therapy and correlates positively with survival.**

Improving homing in T cell therapy

Debora Vignali^a, Marinos Kallikourdis^{a,b,*}

^aAdaptive Immunity Laboratory, Humanitas Clinical and Research Center, Via Manzoni 56, Rozzano (Milano), Italy

^bHumanitas University, Via Manzoni 113, Rozzano (Milano), Italy

T cell migration, search strategies and mechanisms

Matthew F. Krummel, Frederic Bartumeus and Audrey Gérard

T-lymphocyte homing: an underappreciated yet critical hurdle for successful cancer immunotherapy

Robert Sackstein^{1,2,3,4}, Tobias Schatton^{1,3,5,6} and Steven R Barthel^{1,3,5}

Vik Subbu
CEO

- Managing Partner, Equidis Ventures
- Former Director of Business Development, Amplimmune (AZ/MedImmune)
- Strategic Investments, Emergent Biosolutions (EBS)

Ali Tinazli Ph.D.
Scientific Adviser

- Managing Partner, Equidis Ventures
- Head of Life Sciences Strategy, Hewlett Packard
- PhD in BioChemistry from J.W. Goethe University in Germany

Sheng Yao Ph.D.
Scientific Adviser

- SVP of Oncology, Top Alliance Biosciences
- Former Senior Research Scientist and Head, New Target Discovery, Amplimmune
- PhD and Post-doc John Hopkins University

Tonya Webb Ph.D.
Scientific Advisor

- Partner and Chief Executive Officer and Founder, Webbcures
- Associate Professor, Microbiology and Immunology, University of Maryland
- Post-doc John Hopkins University, Immunology

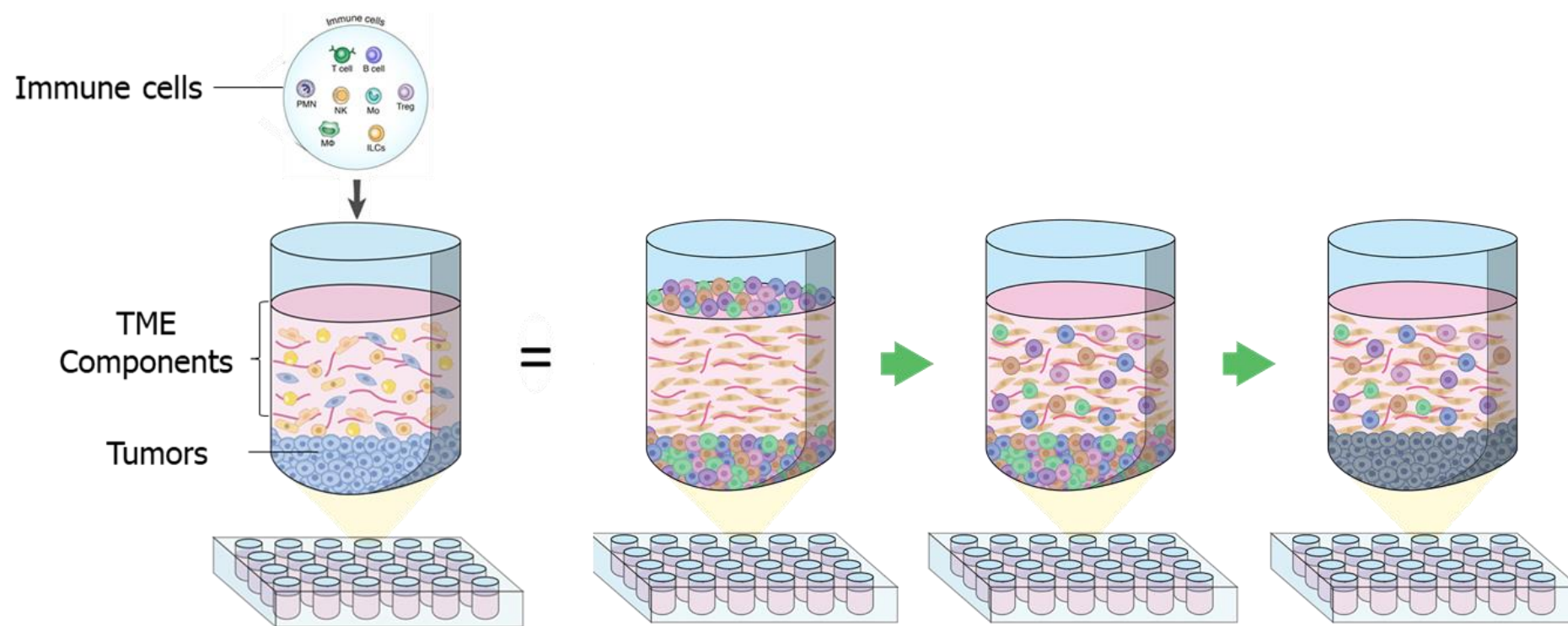
Milton Brown MD
Scientific Advisor

- Director of the Inova Center for Drug Discovery and Development
- Deputy director for Drug Discovery for the Inova Schar Cancer Institute (ISCI)
- MD PhD in Chemistry (Small Molecule Development) and Medicine

Ravi Amaravadi MD
Clinical Advisor

- Partner and Associate Professor of Medicine, University of Pennsylvania
- Expert in Autophagy
- MD, Johns Hopkins University

Physiologically Relevant Platform to Capture the Environments



Simplicity is the ultimate sophistication – Da Vinci

**SIMPLE
DESIGN**

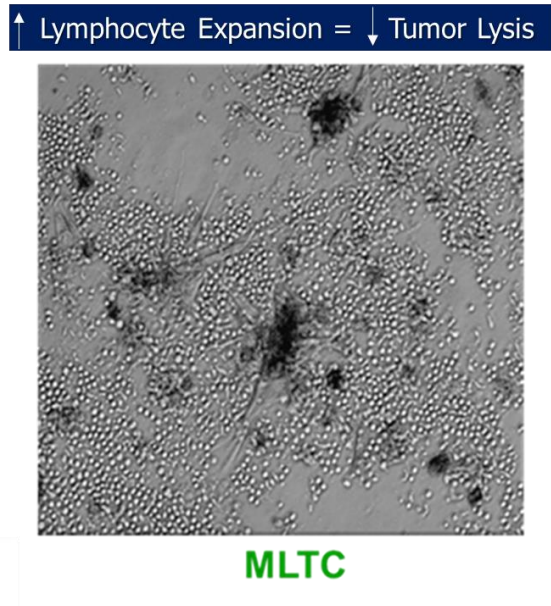
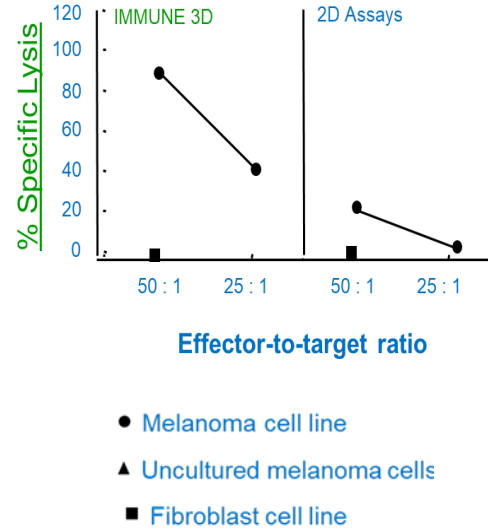
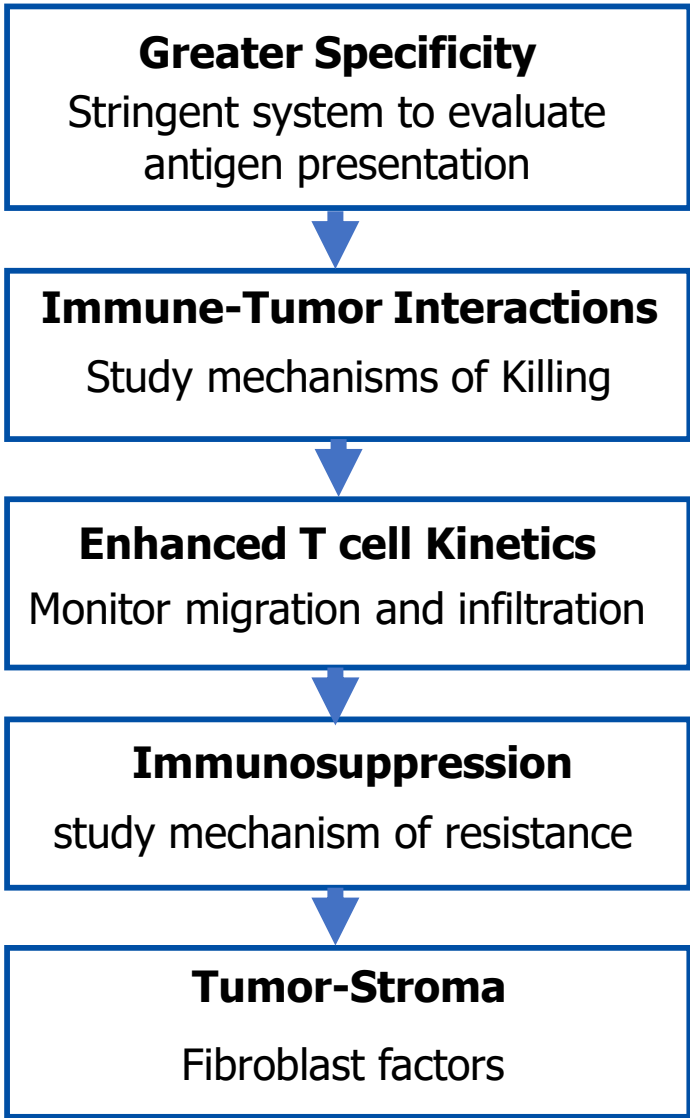
**COST-
EFFECTIVE**

**BROAD
SPECTRUM**

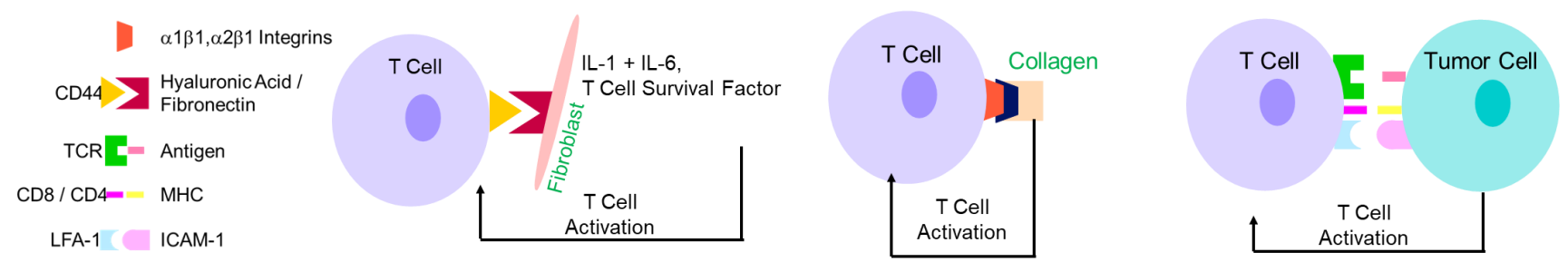
**ISSUED
PATENTED**

**PATIENT
DERIVED**

Platform Advantages



Observed Lymphocyte-Tumor Interactions

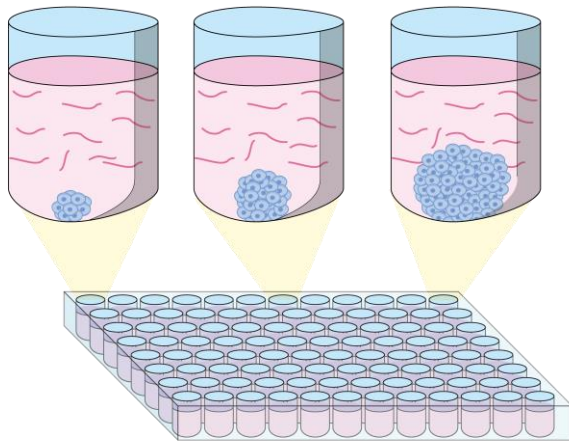


Comparison to 3D Spheroids

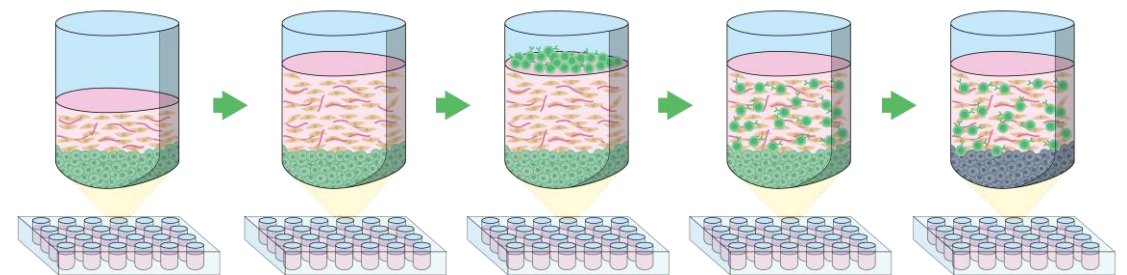
- Uniformity of spheroids
- Not all tumors create spheroids
- Multi-cell evaluations

- ✓ Layered – not spheroid
- ✓ Multi-cell evaluations
- ✓ Migration and Infiltration

Tumor Spheroids

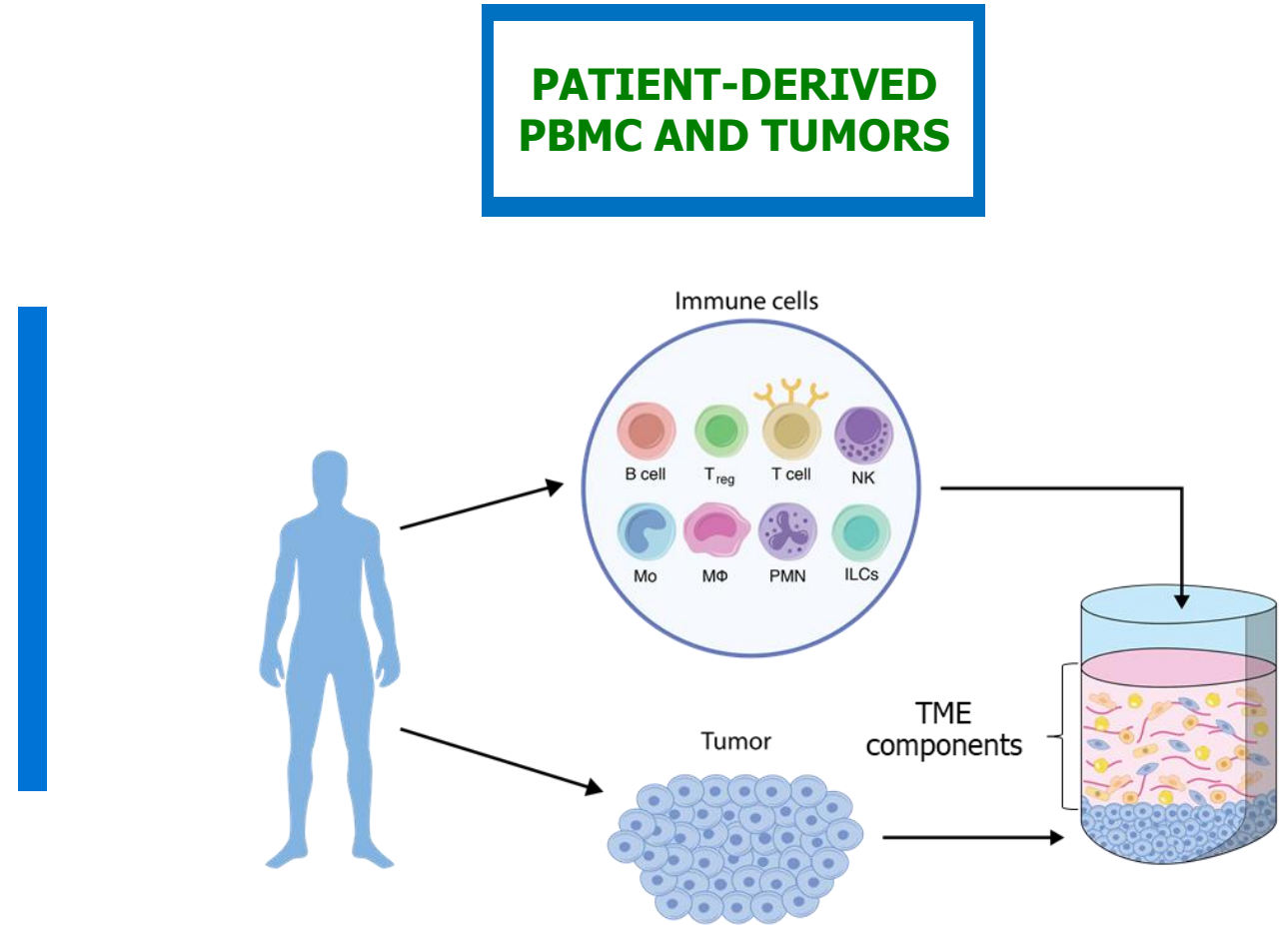
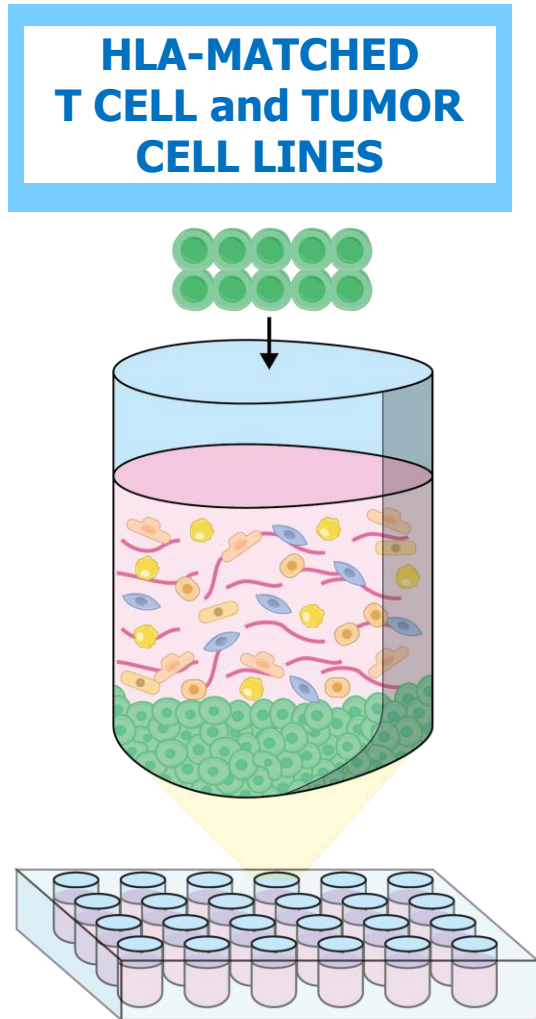


IMMUNE 3D[®]



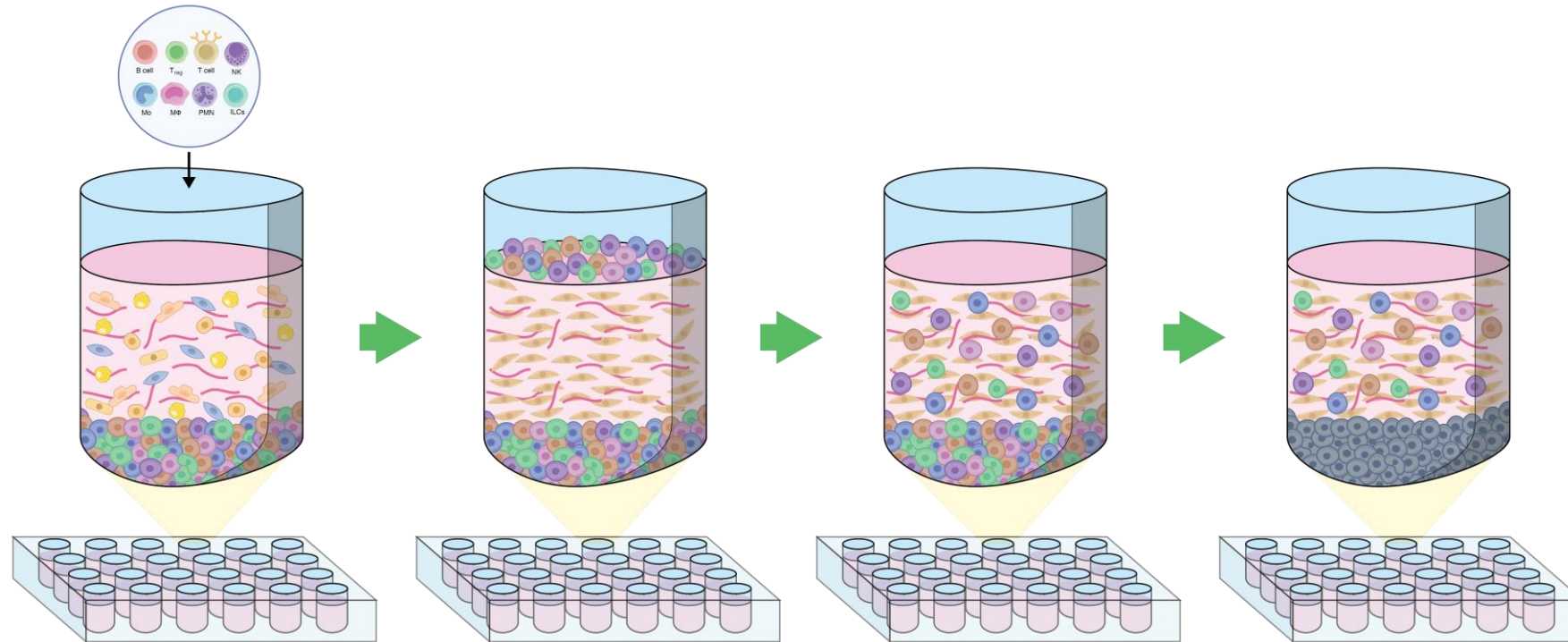
What are the "Inputs"?

Well Characterized, Patient-Derived Cells and Tumor Tissues



How does it work?

HLA-matched cell lines and/or patient-derived samples and tumor tissue are co-cultured at various timepoints in the presence of drug candidates



Screening Outputs

- Mechanism of Action
- Function
- Efficacy

- Toxicity
- Dose
- Combinations

Characterized Patient-Derived HLA-Matched Cell Lines

Re-build the Tumor Environments for Effective Drug Screening and Discovery

PDL1 Sensitive
CTL 001 and
Tumor 001 (colon)

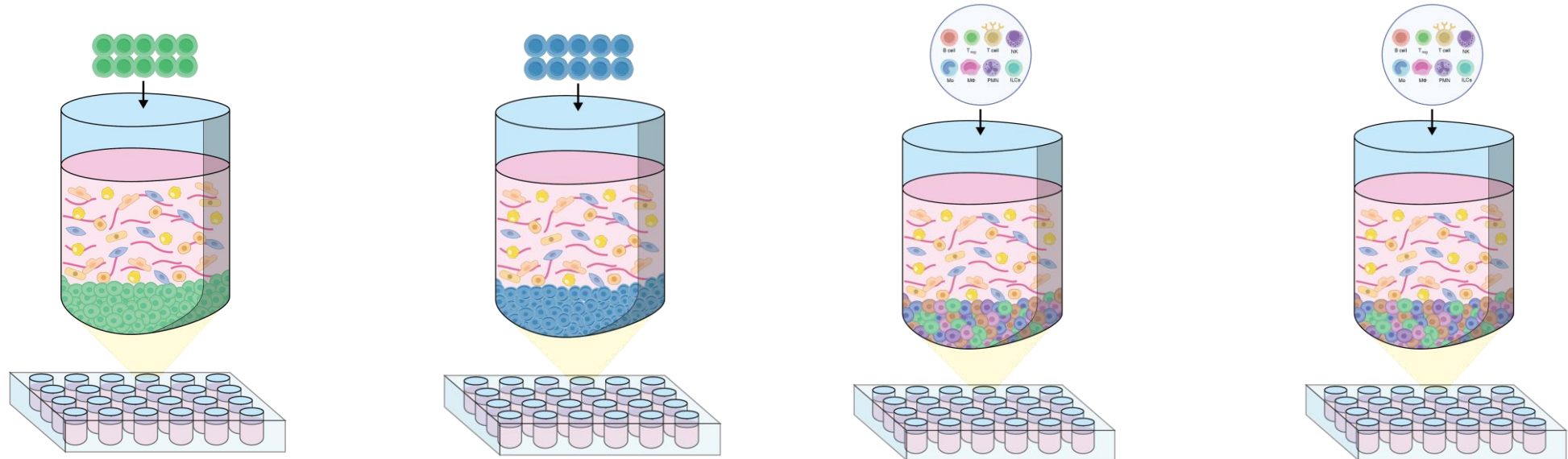
PD1 Sensitive
CTL 003 and
Tumor 003 (mel)

Keytruda Resistant
TIL 004 and
Tumor 004 (mel)

Keytruda Sensitive
TIL 005 and
Tumor 005 (mel)

Advantage

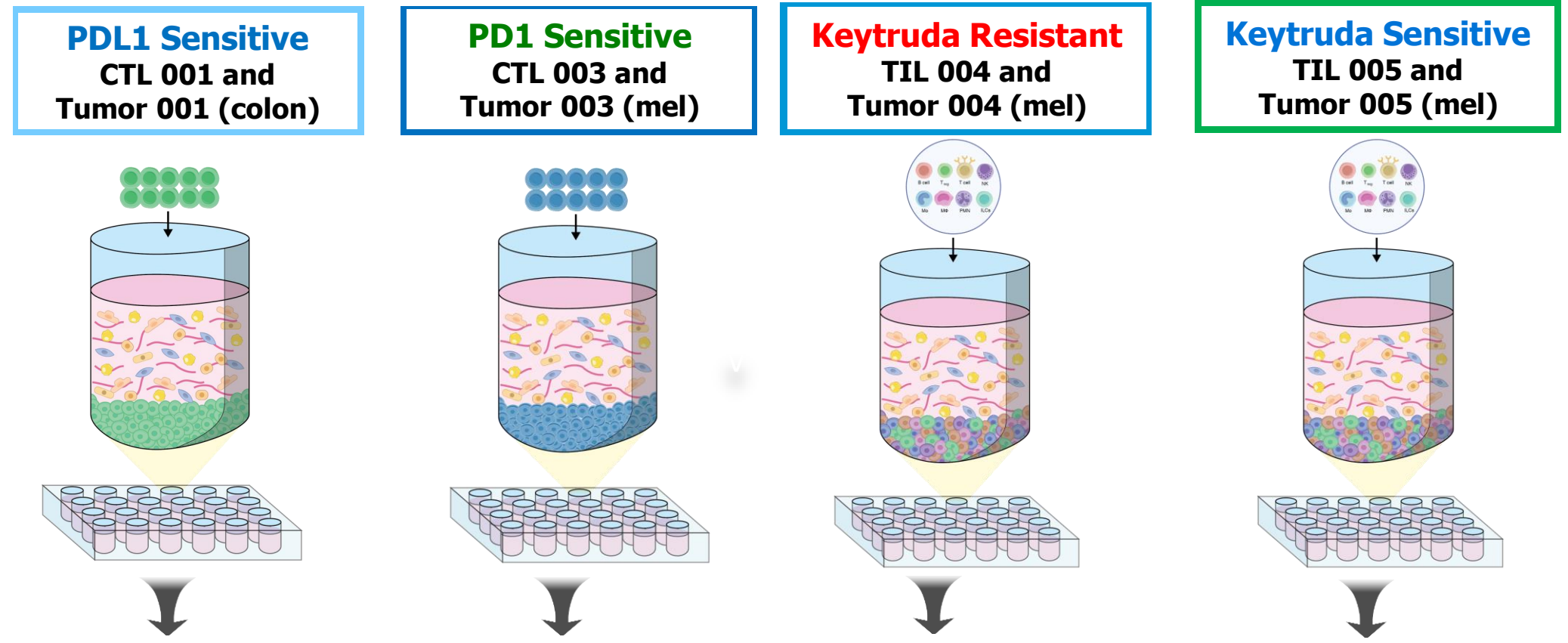
- ✓ Specificity
- ✓ Reproducible
- ✓ Characterized



Extensive characterization, STR profiles and cell surface markers available for each cell line

Rationale for Discovery

Applying our Knowledge and Know-How Using Our Cell Line Platforms

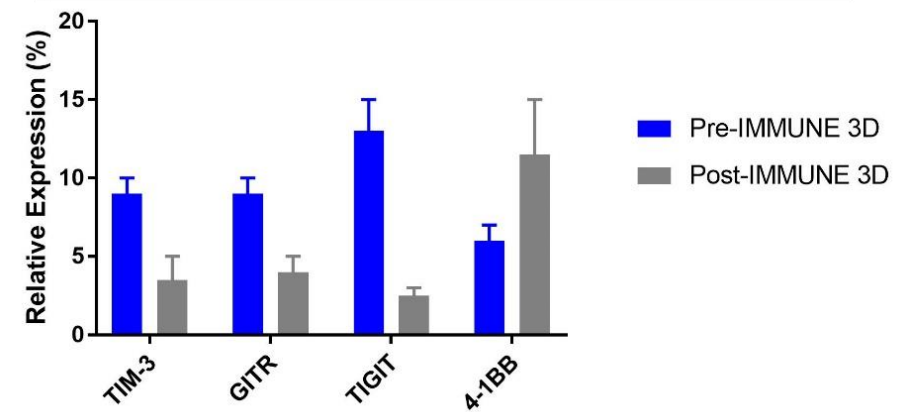
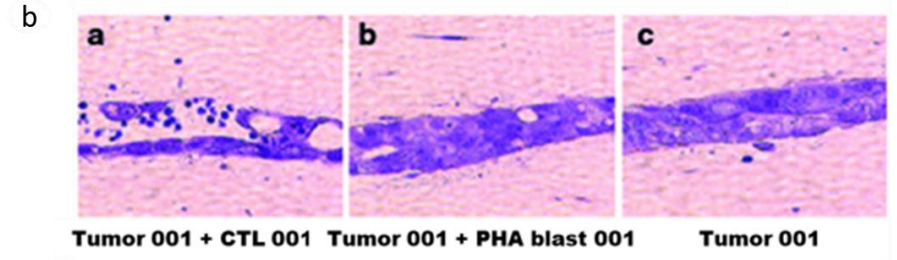
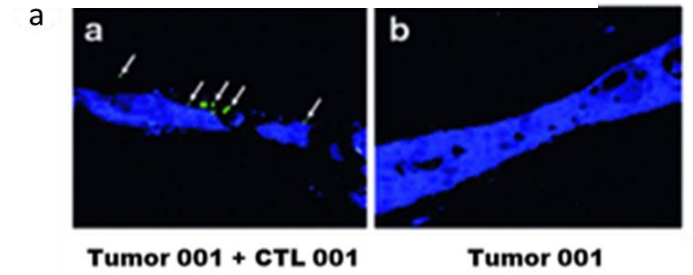
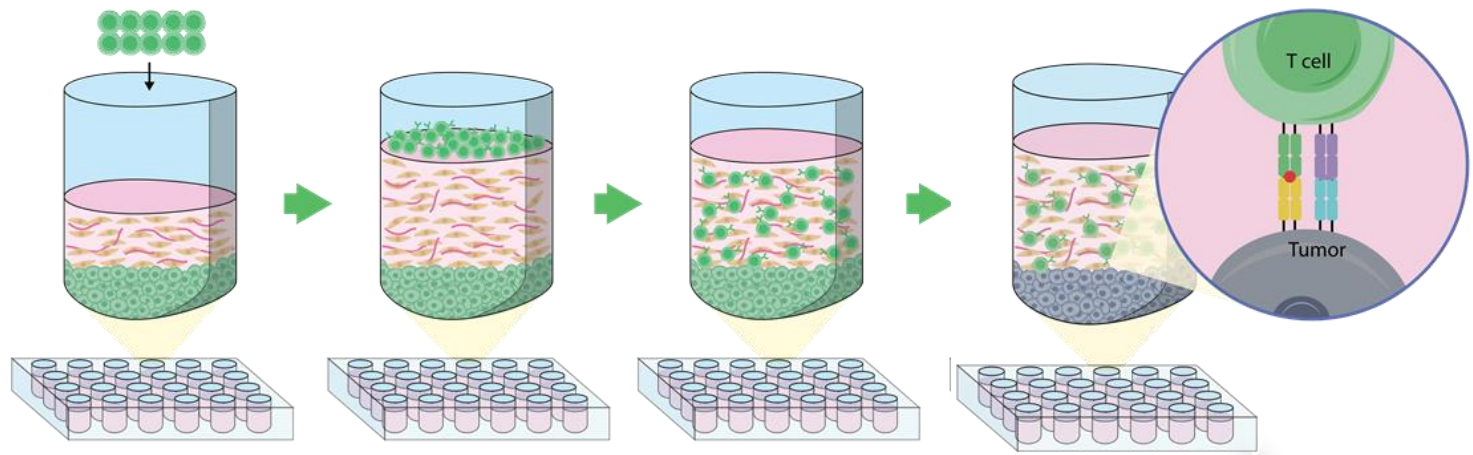


Outputs

- CAR-T
- Antigen
- Stromal Targets
- Epigenetic Targets
- Metabolic
- Combinations

CTL = Cytotoxic T Lymphocyte; TIL = Tumor Infiltrating Lymphocyte

POC for Discovery of Cell Surface Targets



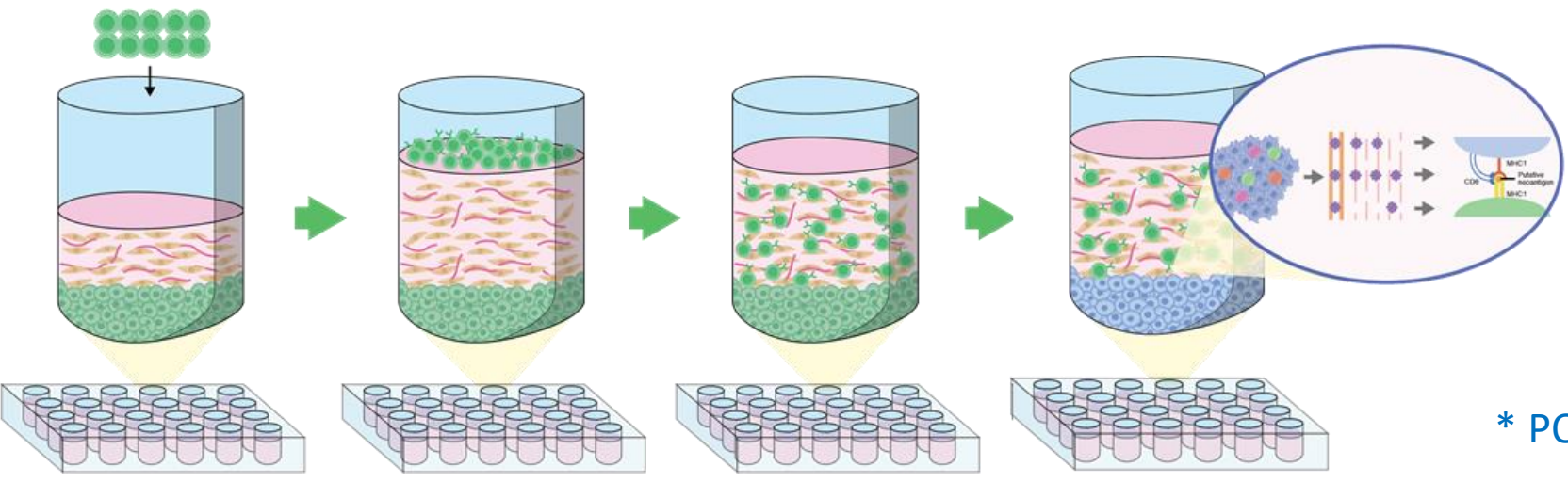
- a. Fluorescent label CTL 001 (green) migration towards Tumor 001 (blue)
- b. H&E staining of CTL 001 lysis of Tumor 001 compared to PHA and No CTL 001
- c. CTL 001 lysis of Tumor 001 (apoptosis %)
- d. Changes of T cell markers pre and post-IMMUNE 3D

Supporting Literature

The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints

Nicolas J. Liosa¹, Michael Cruise^{2,7}, Ada Tam³, Elizabeth C. Wick⁴, Elizabeth M. Hechenbleikner⁴, Janis M. Taube³, Lee Blosser⁴, Hongni Fan¹, Hao Wang⁵, Brandon Luber⁵, Ming Zhang⁶, Nickolas Papadopoulos⁶, Kenneth W. Kinzler⁶, Bert Vogelstein^{6,7}, Cynthia L. Sears^{1,8}, Robert A. Anders², Drew M. Pardoll^{1,2,7,8,*}, and Franck Housseau^{1,*}

POC for Discovery of Neoantigens/Antigens



Discover

- Novel Cancer Antigens*
- T-Cell Receptors
- CAR-T Antigens

* POC data available for antigen discovery

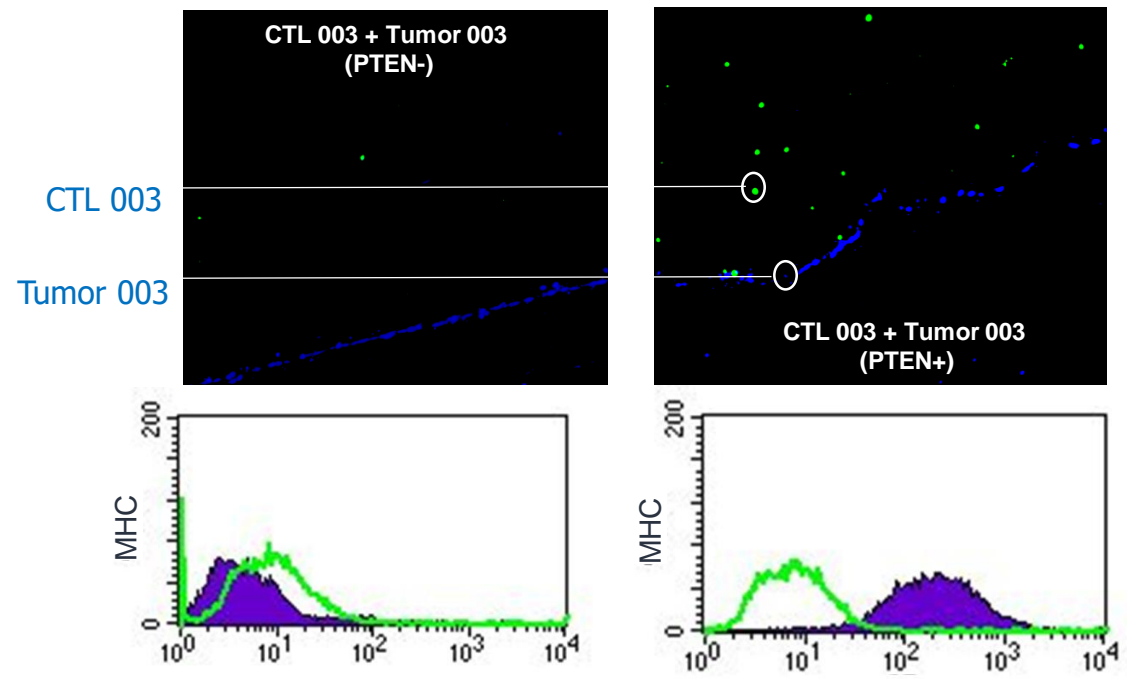
Supporting Literature

Personalized Therapy
Tumor Antigen Discovery for Adoptive Cellular Therapy

Cassian Yee, MD and Gregory A. Lizee, PhD

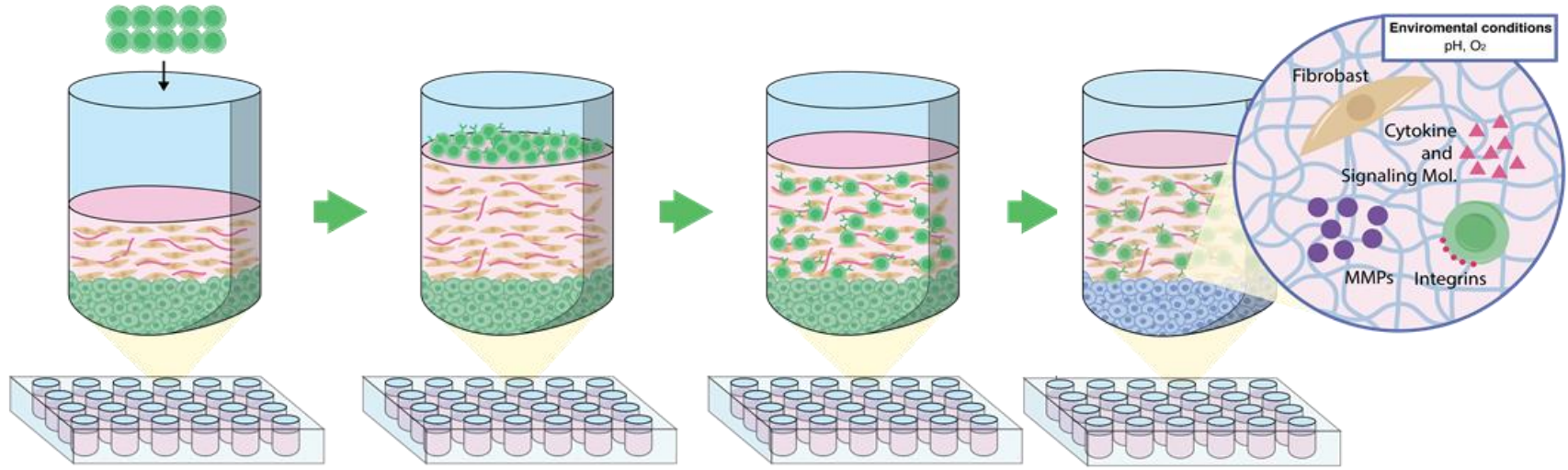
Neoantigens in cancer immunotherapy

Ton N. Schumacher^{1*} and Robert D. Schreiber^{2*}



PTEN mutation in Tumor 003 decreased CTL 003 migration and decreased MHC antigen presentation

POC for Targeting Tumor Stroma



Discover

- Matrix Proteins
- Signaling Targets
- Integrins

Supporting Literature

Can targeting stroma pave the way to enhanced antitumor immunity and immunotherapy of solid tumors?

Ellen Puré and Albert Lo
University of Pennsylvania, Philadelphia, PA, USA

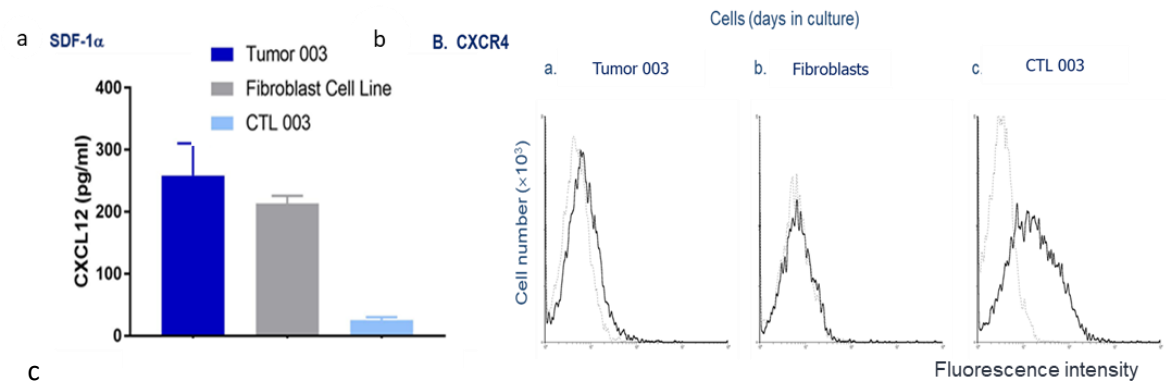
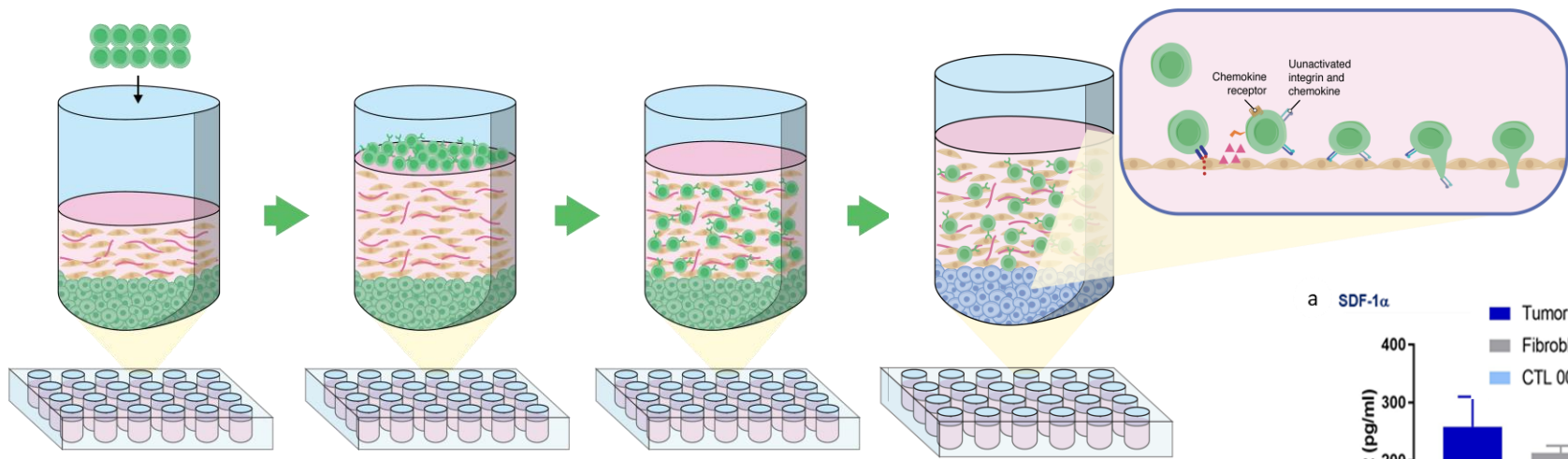
Integrin-targeted cancer immunotherapy elicits protective adaptive immune responses

Byron H. Kwan,^{1,2} Eric F. Zhu,^{2,3} Alice Tzeng,^{1,2} Harun R. Sugito,^{1,2} Ahmed A. Eltahir,^{2,4} Botong Ma,^{2,5} Mary K. Delaney,^{2,3,4} Patrick A. Murphy,^{2,6} Monique J. Kauke,^{2,3} Alessandro Angelini,² Noor Momin,^{1,2} Naveen K. Mehta,^{1,2} Alecia M. Maragh,^{1,2} Richard O. Hynes,^{2,6} Glenn Dranoff,⁷ Jennifer R. Cochran,^{8,9} and K. Dane Wittrup^{1,2,3}

Parameter Investigated	Tumor 003		Tumor 001	
	2D	IMMUNE 3D™	2D	IMMUNE 3D™
CD4	+++	+++	+++	+++
CD8	++	++	+	+
CD40L	-	+	-	-
α1 integrin	-	-	-	-
α2 integrin	-	-	-	-
β1 integrin	+	+++	+++	+++
FAS	++	+++	+++	+++
FASL	-	++	++	-
ICAM-1	+	++	+++	+++
LFA-1	+	+++	+++	+++

- Compared 2D vs. IMMUNE 3D systems using CTL/Tumor 001 (colon) and CTL 003/Tumor 003 (melanoma)
- Certain integrin and adhesion molecules that may contribute to T cell migration are upregulated in IMMUNE 3

POC for Targeting Chemokines



c

Treatment	Total No. of tumor cells Mean \pm SD/field (30 fields)	Percentage of apoptotic tumor cells Mean \pm SD/field (30 fields)
Tumor 003	17.2 \pm 4.5	6.5 \pm 3.4
Tumor 003 + CTL003	11.5 \pm 3.5	19.5 \pm 8.3 ^{a, b}
Tumor 003 + CTL003 + SDF-1 α	15.6 \pm 2.5	5.6 \pm 4.1 ^a
Tumor 003 + CTL003 + AMD3100	18.0 \pm 3.9	5.6 \pm 4.1 ^b
Tumor 003 + CTL003 + control Ig	11.4 \pm 2.8	17.5 \pm 5.2 ^{c, d}
Tumor 003 + CTL003 + α -CXCR4 Ab	13.5 \pm 2.6	4.8 \pm 4.3 ^c
Tumor 003 + CTL003 + a-SDF-1 α Ab	12.7 \pm 2.8	4.9 \pm 4.6 ^d

- a. Expression of SDF-1 α on Tumor 003
- b. Expression of CXCR4 on CTL 003
- c. Effects of blocking CXCR4 and SDF-1 α on apoptosis

Supporting Literature

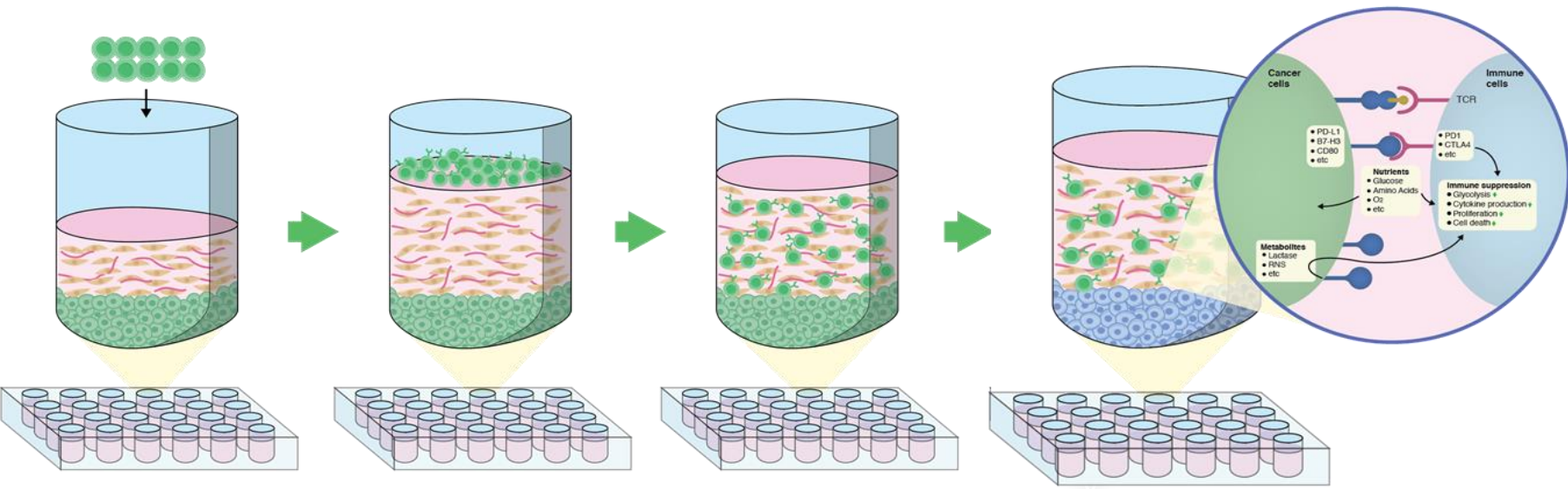
The effects of CCR5 inhibition on regulatory T-cell recruitment to colorectal cancer

S T Ward^{*1}, K K Li², E Hepburn², C J Weston², S M Curbishley², G M Reynolds², R K Hejmadi³, R Bicknell⁴, B Eksteen⁵, T Ismail³, A Rot⁴ and D H Adams²

Regulation of Chemokine Expression in the Tumor Microenvironment

Anton V Gorbachev, Robert L Fairchild

POC for Metabolic Targeting



Discover

- Hypoxic Factors
- IDO
- Metabolic Pathways

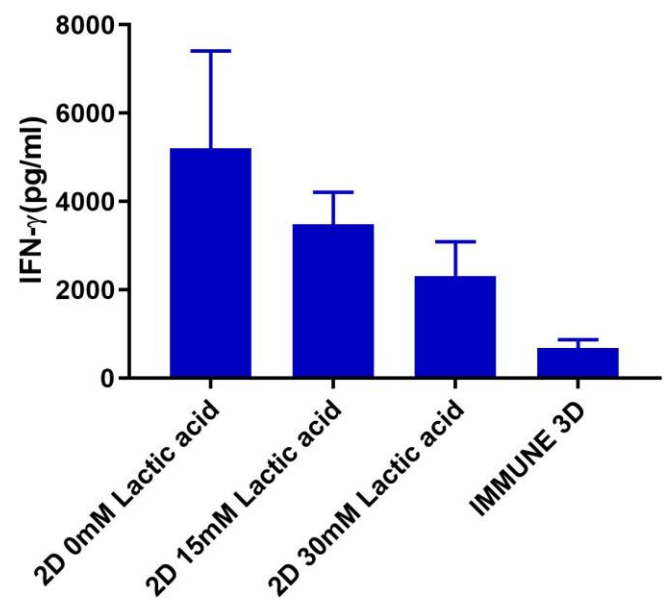
Supporting Literature

Emerging concepts of T cell metabolism as a target of immunotherapy

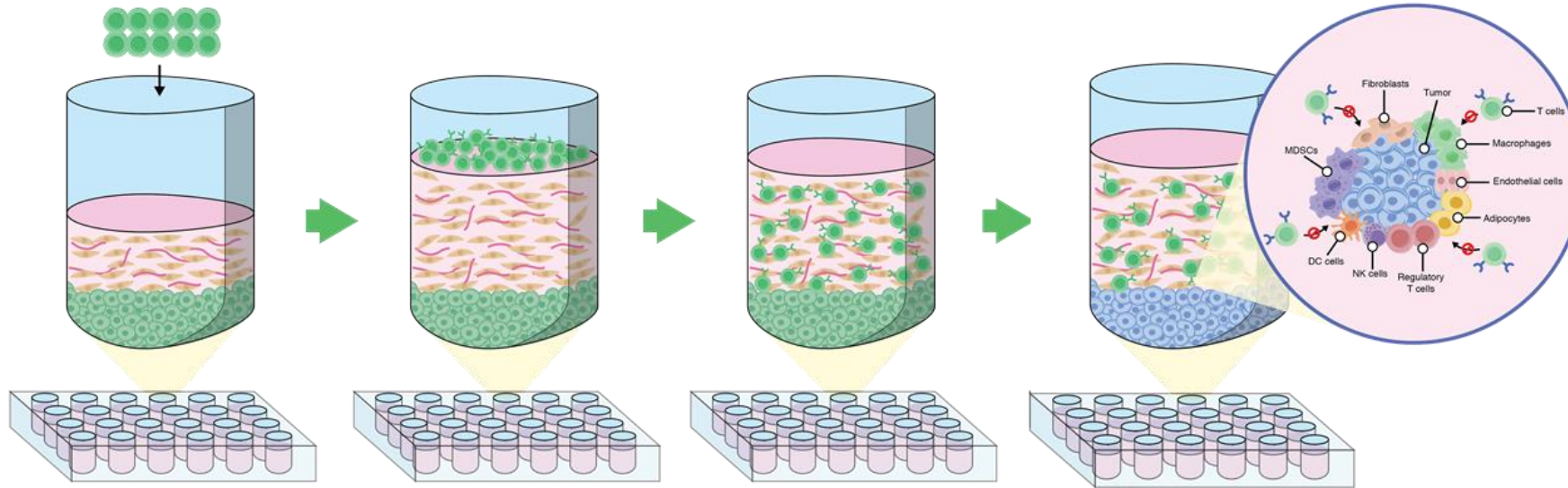
Chih-Hao Chang¹ & Erika L Pearce²

Interplay between Immune Checkpoint Proteins and Cellular Metabolism

Sangbin Lim¹, Joshua B. Phillips¹, Luciana Madeira da Silva¹, Ming Zhou², Oystein Fodstad³, Laurie B. Owen¹, and Ming Tan¹



Using CTL/Tumor 001 (colon) we measured the affect of lactic acid at various concentrations under 2D vs. IMMUNE 3D conditions



Discover

- Myeloid Targets
- Tregs
- Inhibitory Receptors

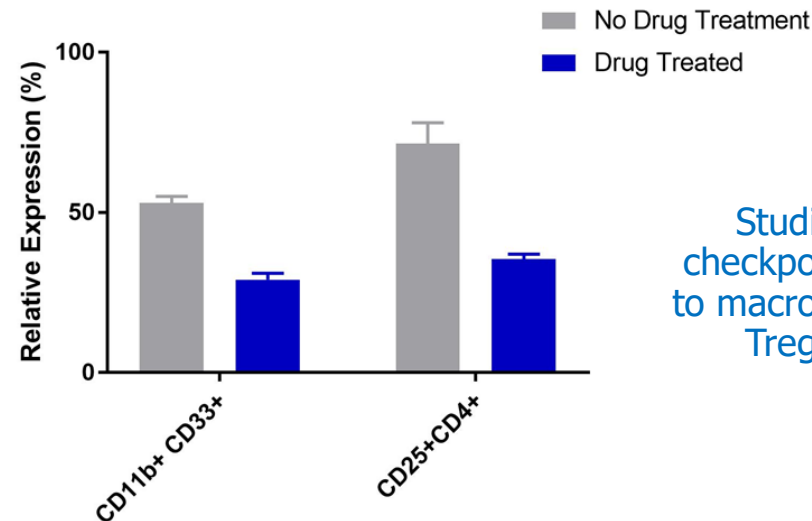
Supporting Literature

Suppression of T Cell Responses in the Tumor Microenvironment

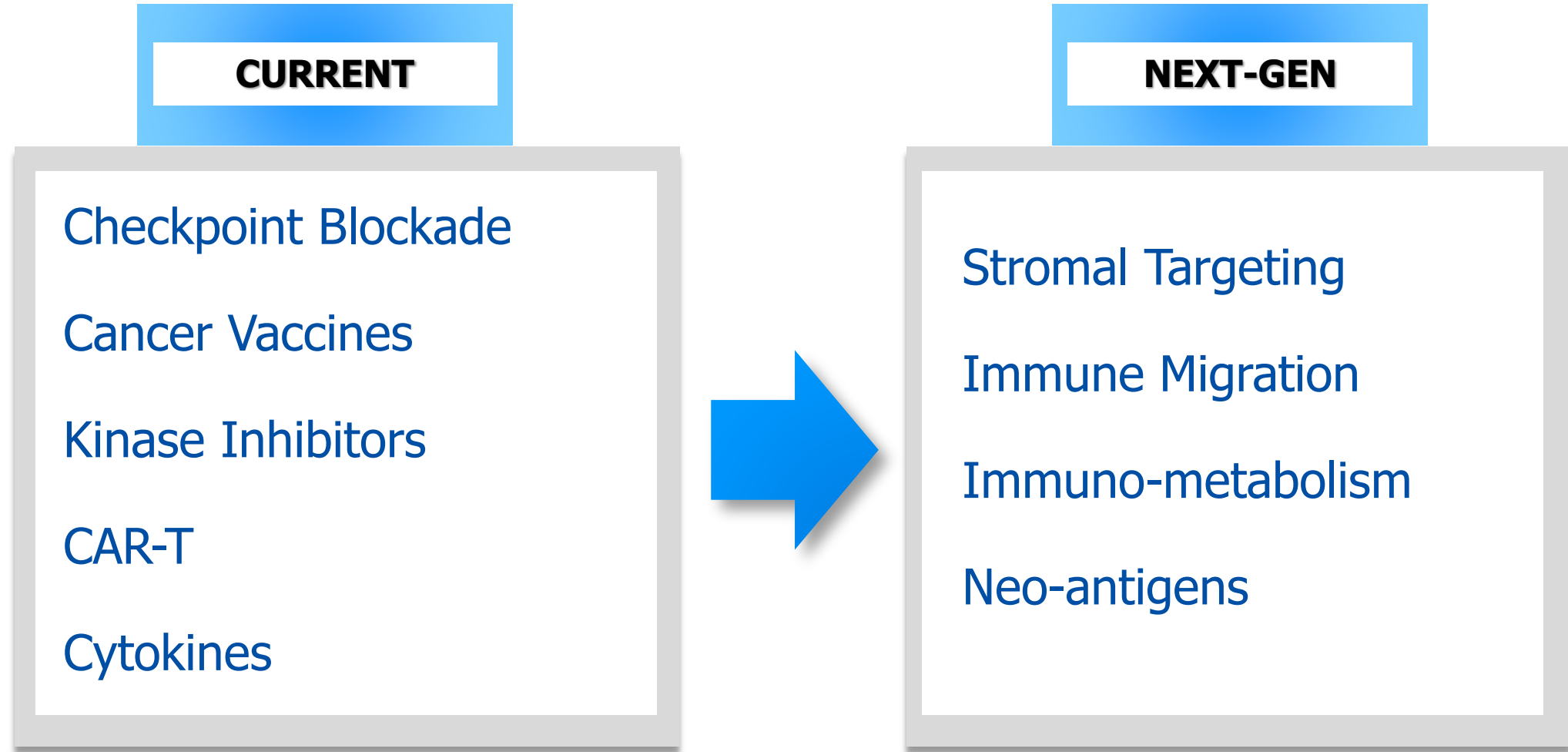
Alan B Frey

Up-Regulation of PD-L1, IDO, and T_{regs} in the Melanoma Tumor Microenvironment Is Driven by CD8⁺ T Cells

Stefani Spranger^{1,*}, Robbert M. Spaapen^{1,*}, Yuanyuan Zha², Jason Williams¹, Yuru Meng¹, Thanh T. Ha¹, and Thomas F. Gajewski^{1,2,†}



Studied the affect of a novel checkpoint drug target on changes to macrophage (CD11b⁺CD33⁺) and Treg markers (CD25⁺CD4⁺)

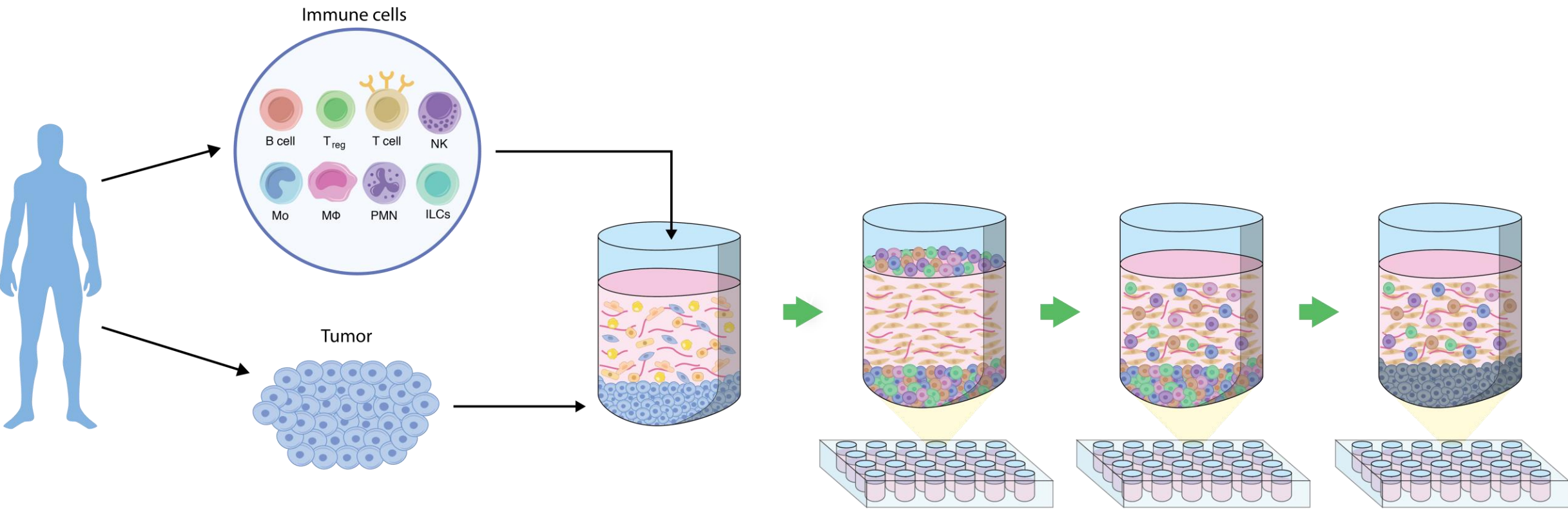


**PROOF-OF-
CONCEPT**

**SIMPLE &
COST EFFECTIVE**

**PATIENT-
DERIVED**

Broad Spectrum IO Discovery Applications



Tumors

- Breast
- Lung
- Ovarian

- Pancreatic
- Prostate
- Bladder

CTL = Cytotoxic T Lymphocyte; TIL = Tumor Infiltrating Lymphocyte



Cells and Assays

Fully functional lab to handle cell expansion, cell culture activities. Liquid NO2 and CO2 equipped lab with continuous monitoring



Techniques

Immune cell isolation and phenotyping
siRNA Knockdown of Gene Expression
SDS-PAGE/Western Blotting
Immunohistochemistry (IHC)
Generation of Stable Expression in Cell Lines
Cloning and Generation of Mutant Proteins
Molecular Biology techniques



People

Experienced professional (ex NIH, academic) with experience in designing assays, developing scopes and delivering results



Instrumentation

Flow cytometry (4 and 16 color)
Perkin Elmer Operetta
Multiplex cytokine panels (Luminex)
Confocal Microscopy
Illumina HiSeq 2500
MiSeqDx
Illumina HiScan system for array analysis.
Targeted RNA analysis or Whole Transcriptome Sequencing
Targeted or Whole Exome Sequencing



Perkin Elmer Operetta

Broad Spectrum IO Discovery Applications

- ✓ Ready-to-Implement IO discovery plans
- ✓ Issued IP - Compositions and Methods of Detecting Cell Migration
- ✓ Lab infrastructure and capabilities for seamless execution
- ✓ Straight forward R&D collaboration
- ✓ Access to leading academic and community hospital partners
- ✓ Due diligence database and term sheet ready

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A New Dimension to 3D Co-Cultures

Simplicity is the ultimate sophistication – Da Vinci

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